HPLC VARIABLES
Guard column: 50 × 6.4 25-37 μm Whatman Co-Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:200 mM ammonium acetate buffer:water 55:10:35
Flow rate: 1
Injection volume: 20
Detector: UV 254

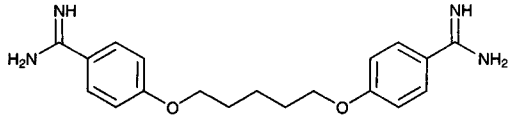
CHROMATOGRAM
Retention time: 15.2
Internal standard: nitroglycerin (8)

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate, saccharin, isosorbide dinitrate

KEY WORDS
tablets; capsules

REFERENCE
Carlson,M.; Thompson,R.D.; Snell,R.P. Determination of isosorbide dinitrate in pharmaceutical products by HPLC, *J.Chromatogr.Sci.*, **1988**, *26*, 574–578.

Pentamidine



Molecular formula: $C_{19}H_{24}N_4O_2$
Molecular weight: 340.43
CAS Registry No.: 100-33-4, 6823-79-6 (dimethanesulfonate), 140-64-7 (isethionate)
Merck Index: 7254

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 4.92 μg/mL hexamidine, vortex briefly, add 500 μL 2 M NaOH, vortex, add 500 μL 2 M HCl, vortex, add 1 mL pH 10 carbonate buffer, vortex, add 4 mL 20 mM di(2-ethylhexyl)phosphoric acid in chloroform, vortex for 1 min, centrifuge at 700 g for 15 min. Remove the chloroform layer and add it to 1 mL 20 mM HCl, vortex for 1 min. Remove the aqueous layer and adjust the pH to 12 with 4 drops 2 M NaOH, add 2 mL dichloromethane, vortex for 1 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL solvent, vortex for 30 s, inject a 20 μL aliquot. (Solvent was MeOH:buffer 50:50. Buffer was 50 mM sodium heptanesulfonate containing 0.4% triethylamine, pH adjusted to 3.0 with phosphoric acid.)

HPLC VARIABLES
Column: 150 × 2.1 5 μm solvent miser C18 (Alltech)
Mobile phase: MeOH:buffer 60:40 (Buffer was 50 mM sodium heptanesulfonate containing 14 mM triethylamine, pH adjusted to 3.0 with phosphoric acid.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.7
Internal standard: hexamidine (8.2)
Limit of detection: 5 ng/mL

KEY WORDS
serum; dog; human; pharmacokinetics

REFERENCE
Dickinson,C.M.; Navin,T.R.; Churchill,F.C. High-performance liquid chromatographic method for quantitation of pentamidine in blood serum, *J.Chromatogr.*, **1985**, *345*, 91–97.

SAMPLE
Matrix: blood
Sample preparation: Condition an SPE-C8 SPE cartridge (Jones Chromatography) with two 1 mL aliquots of MeOH, two 1 mL aliquots of MeCN:1 M pH 3 ammonium acetate 75:25, and five 1 mL aliquots of water, do not allow to dry. 100 μL Plasma + 100 μL water, mix, add to the SPE cartridge, wash with three 1 mL aliquots of MeOH, add 80 μL 500 ng/mL melphalan in MeOH to the SPE cartridge, elute with 1 mL MeCN:1 M pH 3 ammonium acetate 75:25. Evaporate the eluate to dryness under reduced pressure, reconstitute in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:MeOH:triethylamine:200 mM ammonium acetate 18:2:0.5:79.5, pH adjusted to 3.8 with acetic acid
Flow rate: 1.4
Injection volume: 50
Detector: F ex 265 em 345

CHROMATOGRAM
Retention time: 5.5
Internal standard: melphalan (10)
Limit of detection: 8.6 ng/mL

KEY WORDS
plasma; rat; SPE; pharmacokinetics

REFERENCE
Yeh,T.-K.; Dalton,J.T.; Au,J.L.-S. High-performance liquid chromatographic determination of pentamidine in plasma, *J.Chromatogr.*, **1993**, *622*, 255–261.

SAMPLE
Matrix: blood
Sample preparation: Let blood stand at 4° for 4 h, centrifuge at 15000 g for 10 min, add sodium azide to a concentration of 0.01%, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax RX-C8
Mobile phase: Gradient. A was 4.2 mM phosphoric acid containing 10 mM sodium heptanesulfonate and 10 mM tetramethylammonium chloride. B was MeCN:water 75:25 containing 4.2 mM phosphoric acid, 10 mM sodium heptanesulfonate, and 10 mM tetramethylammonium chloride. A:B from 90:10 to 10:90 over 30 min, return to initial conditions over 7 min, re-equilibrate for 3 min.
Flow rate: 1.5
Injection volume: 25
Detector: UV 265

CHROMATOGRAM
Retention time: 18
Internal standard: pentamidine

OTHER SUBSTANCES
Extracted: guanylhydrazones

KEY WORDS
serum; mouse; pentamidine is IS; rat

REFERENCE
Cerami,C.; Zhang,X.; Ulrich,P.; Bianchi,M.; Tracey,K.J.; Berger,B.J. High-performance liquid chromatographic method for guanylhydrazone compounds, *J.Chromatogr.B*, **1996**, *675*, 71–75.

SAMPLE
Matrix: blood, broncho-alveolar lavage fluid, cells, urine
Sample preparation: Plasma. 0.5 mL Plasma + 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge at 1000 g for 10 min, add the supernatant to a Bond Elut C8 SPE cartridge, wash with 1 mL water, wash with 1 mL MeOH:water 50:50, wash with 1 mL MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Bronchoalveolar lavage fluid. Centrifuge bronchoalveolar lavage fluid, add 1 mL supernatant to 1 mL Sorensen's buffer and 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge, add the supernatant to a Bond Elut C8 SPE cartridge, wash with water, wash with MeOH:water 50:50, wash with MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Alveolar cells. Wash alveolar cells (from 10 mL bronchoalveolar lavage fluid) twice with phosphate buffered saline, resuspend in 1 L Sorensen's buffer, vortex for 1 min. 250 μL Suspension + 250 μL Sorensen's buffer + 1 mL 30 ng/mL hexamidine in MeCN, vortex, centrifuge, add the supernatant to a Bond Elut C8 SPE cartridge, wash with water, wash with MeOH:water 50:50, wash with MeOH, elute with MeOH:water:sodium heptanesulfonate:tetramethylammonium chloride:phosphoric acid 97.2:2.2:0.5:0.02:0.1, evaporate the eluate to 200 μL under a stream of nitrogen, inject a 50 μL aliquot. Urine. 200 μL Urine + 1 mL 750 ng/mL hexamidine in MeCN, vortex for 1 min, centrifuge at 1000 g for 5 min, inject a 20 μL aliquot (J.Infect.Dis. 1986, 154, 823).

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Octyl 5
Mobile phase: MeCN:water 21:79 containing 0.02% tetramethylammonium chloride and 0.1% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: F ex 275 em 340

CHROMATOGRAM
Internal standard: hexamidine
Limit of detection: 2.29 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Conte,J.E.,Jr.; Golden,J.A. Intrapulmonary and systemic pharmacokinetics of aerosolized pentamidine used for prophylaxis of *Pneumocystis carinii* pneumonia in patients infected with the human immunodeficiency virus, *J.Clin.Pharmacol.*, **1995**, *35*, 1166–1173.

SAMPLE
Matrix: cells
Sample preparation: 400 μL Cells in phosphate saline glucose (99:1) buffer + 10 μL 10% orthophosphoric acid, vortex, filter (Ultrafree-MC polysulfone membrane, 100000 molecular mass cut-off) while centrifuging at 5000 g for 5 min, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm pellicular Spherisorb RP-18
Column: 200 × 2 5 μm Nucleosil C18
Mobile phase: MeOH:5 mM citric acid 50:50 containing 5 mM sodium pentanesulfonate, pH adjusted to 4.0 with NaOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 261

CHROMATOGRAM
Retention time: 6
Limit of detection: 5.7 ng/mL

REFERENCE
Rabanal,B.; De Arriba,R.G.; Garzón,M.J.; Reguera,R.M.; Balaña-Fouce,R.; Negro,A. Determination of pentamidine in *Leishmania* infantum promastigotes by ion-paired liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 2017–2029.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 6 mL microsomal incubation + 500 μL MeCN + hexamidine, add to a Prep-Sep C18 SPE cartridge (Fisher), wash with water, wash with MeCN, elute with 1 mL MeCN: buffer 95:5. Evaporate the eluate to near dryness under a stream of air, reconstitute the residue in 200 μL mobile phase, inject a 5 μL aliquot. (Buffer was 4.2 mM phosphoric acid containing 10 mM heptanesulfonate and 10 mM tetramethylammonium chloride.)

HPLC VARIABLES
Column: 250 X 4.6 Zorbax 5 μm RX diisopropyl C8
Mobile phase: Gradient. MeCN:buffer from 22.5:77.5 to 45:55 over 25 min (Buffer was 4.2 mM phosphoric acid containing 10 mM heptanesulfonate and 10 mM tetramethylammonium chloride.)
Column temperature: 40
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 16
Internal standard: hexamidine

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver; SPE

REFERENCE
Berger,B.J.; Reddy,V.V.; Le,S.T.; Lombardy,R.J.; Hall,J.E.; Tidwell,R.R. Hydroxylation of pentamidine by rat liver microsomes, *J.Pharmacol.Exp.Ther.*, **1991**, *256*, 883–889.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Lyophilize microsomal incubation. Dissolve lyophilizate in 150 μL mobile phase, mix thoroughly for 5 min, centrifuge at 6000 g for 5 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B
Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: Gradient. A was 10 mM sodium octylsulfonate, 10 mM tetramethylammonium chloride, and 20 mM phosphoric acid adjusted to pH 3.0 with ammonia. B was MeOH. A:B from 60:40 to 30:70 over 30 min.
Flow rate: 1
Injection volume: 15
Detector: UV 260

CHROMATOGRAM
Retention time: 22
Limit of detection: 0.02 nmole

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rabbit; liver

REFERENCE
Clement,B.; Jung,F. *N*-Hydroxylation of the antiprotozoal drug pentamidine catalyzed by rabbit liver cytochrome P-450 2C3 or human liver microsomes, microsomal retroreduction, and further oxidative transformation of the formed amidoximes Possible relationship to the biological oxidation of arginine to NG-hydroxyarginine, citrulline, and nitric oxide, *Drug Metab.Dispos.*, **1994**, *22*, 486–497.

SAMPLE
Matrix: urine
Sample preparation: Directly inject an aliquot of urine. Alternatively add urine to a Sep-Pak SPE cartridge, elute with 2 mL MeCN:water 50:50, inject an aliquot of the eluate.

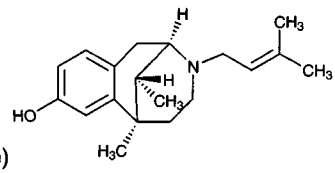
HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 6 ammonium acetate buffer:triethylamine 3:96.8:0.2. B was MeCN:50 mM pH 6 ammonium acetate buffer:triethylamine 50:49.8:0.2. A:B from 100:0 to 0:100 in 60 min (?)
Injection volume: 100-150
Detector: MS, Finnigan TSQ 700, chemical ionization APCI interface, vaporizer 400°, heated capillary 200°, current 5 μA, m/z 357 (The effluent from the column was directed to waste for 2 min, then to the MS.)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; SPE

REFERENCE
Nordin,J.; Wilkström,I.; Bronner,U.; Gustafsson,L.L.; Ericsson,. Liquid chromatography-tandem mass spectrometry applied to a study of the metabolism of pentamidine. Discussion of possibilities and problems, *J.Chromatogr.A*, **1997**, *777*, 73–79.

Pentazocine



Molecular formula: $C_{19}H_{27}NO$
Molecular weight: 285.45
CAS Registry No.: 359-83-1, 64024-15-3 (HCl), 17146-95-1 (lactate)
Merck Index: 7261
Lednicer No.: 1 297; 2 325

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8

Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 7.4
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, meperidine, methadone, normeperidine, norpropoxyphene, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 1 M NaOH + 6 mL 20 ng/mL levallorphan tartrate in diethyl ether, agitate at 4° for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 250-500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Nucleosil RP 18
Mobile phase: MeCN:5 mM phosphoric acid 33:67
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 324

CHROMATOGRAM
Retention time: 6.2
Internal standard: levallorphan tartrate (4.9)
Limit of detection: 1 ng/mL
Limit of quantitation: 4 ng/mL

KEY WORDS
plasma

REFERENCE
Moeller,N.; Dietzel,K.; Nuernberg,B.; Geisslinger,G.; Brune,K. High-performance liquid chromatographic determination of pentazocine in plasma, *J.Chromatogr.*, **1990**, *530*, 200–205.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES

Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: Gradient A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.50 ppm

OTHER SUBSTANCES

Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, procaine
Also analyzed: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam, oxazepam

KEY WORDS

whole blood

REFERENCE

Bernal,J.L.; Del Nozal,M.J.; Rosas,V.; Villarino,A. Extraction of basic drugs from whole blood and determination by high performance liquid chromatography, *Chromatographia*, **1994**, *38*, 617–623.

SAMPLE

Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 1.9
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, promazine, propafenone, propoxyphene, protriptyline, quinidine, temazepam, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol,

MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: encainide, lidocaine, propranolol

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 4.33
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine;

bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents

Sample preparation: 1 mL Whole blood or gastric contents + 50 μL 400 μg/mL IS in MeOH + 1 mL EtOH + 5 drops 1 M pH 9 potassium carbonate + 2 mL water + 8 mL n-hexane:MTBE 25:75, rotate for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil BDS C18

Mobile phase: Gradient. A was MeCN:MeOH:1.5 M ammonium acetate:water 10:10:3:77. B was MeCN:MeOH:1.5 M ammonium acetate:water 40:40:3:17. A:B from 95:5 to 50:50 over 20 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 15

Internal standard: N-allylnormetazocine (Sigma) (9)

OTHER SUBSTANCES

Extracted: zopiclone

KEY WORDS

whole blood

REFERENCE

Van Bocxlaer,J.; Meyer,E.; Clauwaert,K.; Lambert,W.; Piette,M.; De Leenheer,A. Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection, *J.Anal.Toxicol.*, **1996**, *20*, 52–54.

SAMPLE

Matrix: blood, saliva, tissue, urine

Sample preparation: Homogenize (Polytron) tissue with 4 (whole brain) or 8 (brain striata) volumes of 100 mM pH 4.5 NaH_2PO_4 containing 0.5% NaF. Add 500 μL brain homogenate or 500 μL plasma, saliva, or urine containing 15 μL saturated NaF solution to 75 μL 150 μg/mL IS, add 50 μL 50% perchloric acid, mix vigorously for 10 s, let stand at room temperature for 10 min, add 1 mL water, mix briefly, centrifuge at 10° at 2500 (?) for 30 min. Remove the supernatant and add it to 750 μL saturated sodium carbonate solution, mix briefly, add 7.5 mL pentane:chloroform 95:5, rock gently for 10 min, centrifuge in a desk-top centrifuge for 2 min, freeze in dry ice/acetone for 2 min. Remove the organic layer and add it to 250 μL 100 mM HCl, mix vigorously for 10 s, centrifuge in a desk-top centrifuge for 1-2 min, freeze in dry ice/acetone for 3-5 min, discard the organic layer. Allow the aqueous layer to thaw, remove any trace of organic solvent with a stream of nitrogen, inject a 75 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee RP-8

Column: 250 × 4.6 5 μm Zorbax RX-C18
Mobile phase: MeCN:buffer 18:82 (Buffer was 100 mM K_2HPO_4 containing 0.5% triethylamine, adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 2
Injection volume: 75
Detector: UV 235

CHROMATOGRAM
Retention time: 11.5
Internal standard: 2β-carbomethoxy-3β-(4-chlorophenyl)tropane (RTI-31) (Research Biochemical International, Natick MA) (11.4)

OTHER SUBSTANCES
Extracted: chlordiazepoxide, clozapine, cocaine, gepirone, methylphenidate, pseudococaine
Simultaneous: acetaminophen, acetophenazine, amoxapine, amphetamine, atropine, benperidol, buspirone, caffeine, carbamazepine, chlorpheniramine, codeine, dextromethorphan, diazepam, diphenhydramine, flupenthixol, flurazepam, haloperidol, hydergine, hydrocodone, hydromorphone, lidocaine, loxapine, mepazine, meperidine, mesoridazine, methaquaalude, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, 3,4-methylenedioxymethamphetamine, morphine, norcocaine, oxazepam, pentobarbital, phenylpropanolamine, procainamide, procaine, propyl benzoylecgonine, quinidine, quinine, salicylic acid, secobarbital, theophylline, trazodone, 3-tropanyl-3,5-dichlorobenzoate, vancomycin, WIN 35428
Noninterfering: amitriptyline, benztropine methanesulfonate, butaperazine, butriptyline, carphenazine, chlorpromazine, clomipramine, cyclobenzaprine, dextropropoxyphene, dronabinol, ephedrine, ethchlorvynol, fluoxetine, fluphenazine, imipramine, meprobamate, methadone, methamphetamine, nicotine, norfluoxetine, nortriptyline, PCP, phenothiazine, pseudoephedrine

KEY WORDS
rat; cow; plasma; brain

REFERENCE
Bonate,P.L.; Davis,C.M.; Silverman,P.B.; Swann,A. Determination of cocaine in biological matrices using reversed phase HPLC: Application to plasma and brain tissue, *J.Liq.Chromatogr.*, **1995**, *18*, 3473–3494.

SAMPLE
Matrix: blood, tissue, vitreous humor
Sample preparation: Blood, vitreous humor. Mix 1 mL sample with 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 200 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer. Tissue. Mix 500 μL liver homogenate with 500 μL 2% pH 9.5 sodium tetraborate and 8 mL n-butanol:hexane 5:95. Extract for 30 min, centrifuge. Remove the organic layer and add it to 400 μL 0.2% orthophosphoric acid. Extract for 30 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak-Phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 55:45, adjusted to pH 3.0
Flow rate: 1.5
Injection volume: 30
Detector: UV 214

OTHER SUBSTANCES
Extracted: pimozide, sertraline

KEY WORDS
liver; pentazocine is IS

REFERENCE
McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 12.522

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN

Mobile phase: MeOH:3 mM ammonium acetate 90:10

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS

tablets; syrups; elixirs; injections

REFERENCE

Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.21

OTHER SUBSTANCES
Simultaneous: thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, normethadone, meperidine, dipipanone, diamorphine, acetylcodeine, monoacetylmorphine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipa-

none, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.91 (A), 5.47 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, met-

ronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.8

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

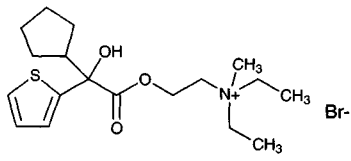
Extracted: nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine

Interfering: amphetamine, phenmetrazine, lidocaine, ephedrine, methamphetamine, desipramine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

Penthienate bromide



Molecular formula: $C_{18}H_{30}BrNO_3S$
Molecular weight: 420.41
CAS Registry No.: 60-44-6
Merck Index: 7268

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

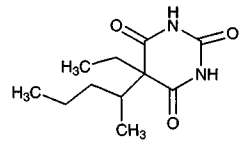
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, pri-

maquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Pentobarbital



Molecular formula: $C_{11}H_{18}N_2O_3$
Molecular weight: 226.28
CAS Registry No.: 76-74-4, 57-33-0 (Na salt)
Merck Index: 7272
Lednicer No.: 1 269

SAMPLE

Matrix: blood
Sample preparation: 200 µL Serum + 200 µL 50 µg/mL hexobarbital in MeCN + 25 µL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 µL aliquot of the supernatant.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM

Retention time: 22.4
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, mesantoin, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine

KEY WORDS

serum

REFERENCE

Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 1 mL buffer, vortex, add 10 mL n-butyl chloride containing 10 μg/mL barbital and 4 μg/mL thiamylal, extract vigorously for 3 min, centrifuge at 3000 g for 5 min. Remove the upper organic layer and add it to 100 μL 450 mM NaOH, extract vigorously for 3 min, centrifuge for 10 min or until lower aqueous phase is clear, inject a 15 μL aliquot of the lower aqueous phase. (Soak glassware in 1 M HCl overnight, rinse with water, dry. Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 2.4
Internal standard: barbital (0.8), thiamylal (5.2)
Limit of detection: 2 μg/mL

OTHER SUBSTANCES
Extracted: thiopental
Simultaneous: acetaminophen, acetazolamide, aspirin, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid
Interfering: amobarbital

KEY WORDS
serum

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: blood
Sample preparation: 50 μL 25 μg/mL 5-Ethyl-5-p-tolylbarbituric acid in MeOH added to 150 × 10 mm glass centrifuge tube and blow dry under a stream of nitrogen, add 500 μL plasma, add 5 mL dichloromethane, mix on rotary mixer for 5 min, centrifuge. Remove organic layer, evaporate to dryness under nitrogen, take up in 500 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 μBondapak C18 Corasil B
Column: 300 × 4 5 μm μBondapak C18
Mobile phase: MeOH:10 mM potassium phosphate adjusted to pH 4.40 ± 0.05 with 150 mM phosphoric acid 50:50
Flow rate: 1.7
Injection volume: 40
Detector: UV 212

CHROMATOGRAM
Retention time: 8.90
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (4.62)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: thiopental (UV 284)

Simultaneous: acetaminophen, amobarbital, barbital, butalbital, butabarbital, caffeine, carbamazepine, phenacetin, phenobarbital, phenytoin, secobarbital, theobromine, theophylline, vinbarbital

KEY WORDS
plasma

REFERENCE
Houdret,N.; Lhermitte,M.; Lalau,G.; Izydorczak,J.; Roussel,P. Determination of thiopental and pentobarbital in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 437–442.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. 500 μL Plasma + 10 μL 1 mg/mL sodium secobarbital in EtOH, add to the SPE cartridge, wash with 2 volumes of 100 mM pH 5.59 Sörensen's phosphate buffer, wash with 1 volume of water, elute with 500 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.21
Internal standard: secobarbital (6.39)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: methohexital, thiopental
Noninterfering: ketamine

KEY WORDS
plasma; dog; pharmacokinetics; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 5.86
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Simultaneous: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

KEY WORDS
serum

REFERENCE
Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE
Matrix: blood
Sample preparation: Vigorously shake equal volumes of plasma and MeCN, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 110 × 4.6 PartiSphere C8 (Whatman)
Mobile phase: MeCN:120 mM pH 6.2 phosphate buffer 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted PTFE coil irradiated with a Sylvania G8-T5 lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 3.28
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma

REFERENCE
Schmid,R.W.; Wolf,C. Simultaneous determination of thiopental and its metabolite, pentobarbital, in blood by high-performance liquid chromatography and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1749–1755.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 9.18
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS
plasma; SPE

REFERENCE
Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE
Matrix: blood
Sample preparation: 200-500 μL Whole blood + 1 mL 100 mM pH 7.5 phosphate buffer, vortex for 1 min, add 7 mL n-hexane:diethyl ether 50:50, add 50 μL 100 μg/mL secobarbital in EtOH:water 75:25, shake for 15 min, centrifuge at 4° at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, vortex, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil C18
Mobile phase: MeCN:water 32:68
Flow rate: 0.3
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: secobarbital (9)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
whole blood

REFERENCE
Celardo,A.; Bonati,M. Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 220–225.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with an equal volume of MeCN, centrifuge at 10000 g, dilute supernatant with an equal volume of water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 110 × 4.7 5 μm PartiSphere C18 (Whatman)
Mobile phase: MeCN:15 mM pH 7.0 phosphate buffer 30:70
Flow rate: 0.8
Injection volume: 50
Detector: UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted coil of PTFE tubing irradiated by an 8 W low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: aprobarbital, butethal, secobarbital

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Wolf,C.; Schmid,R.W. Enhanced UV-detection of barbiturates in HPLC analysis by on-line photochemical reaction, *J.Liq.Chromatogr.*, **1990**, *13*, 2207–2216.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 20 μL 500 μg/mL phenylbutazone in 2 mM NaOH + 1.1 mL ether:n-hexane 20:80 + 20 μL 3 M phosphoric acid, vortex at 1200 rpm for 1 min, centrifuge at 2000 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure (16 mbar) at 40° for 10 min, reconstitute the residue in 50 μL 400 μM NaOH, inject a 2-40 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 AGP (ChromTech)
Column: 100 × 4 AGP-CSP (ChromTech)
Mobile phase: Isopropanol:100 mM pH 6.2 phosphate buffer 4.5:95.5
Flow rate: 0.9
Injection volume: 2-40
Detector: UV 220

CHROMATOGRAM
Retention time: 3.1 (R(+)), 4.1 (S(-))
Internal standard: phenylbutazone (15.7)

OTHER SUBSTANCES
Extracted: thiopental (UV 287)

KEY WORDS
sheep; plasma; chiral

REFERENCE
Huang,J.L.; Mather,L.E.; Duke,C.C. High-performance liquid chromatographic determination of thiopentone enantiomers in sheep plasma, *J.Chromatogr.B*, **1995**, *673*, 245–250.

SAMPLE
Matrix: blood

Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 55:45
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM

Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Also analyzed: thiopental

KEY WORDS

serum; rat; pharmacokinetics

REFERENCE

Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 12.12
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 1.50
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: moclobemide, tranylcypromine, metoclopramide

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.437

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve injection in mobile phase to give a pentobarbital sodium concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Partisil ODS-3 or 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer:propylene glycol 55:45:4 (Buffer was 4.1 g anhydrous sodium acetate and 15 mL acetic acid in 1 L water.)
Flow rate: 2
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rugged; injections

REFERENCE
Reif,V.D.; Kaufmann,K.L.; DeAngelis,N.J.; Frankhouser,M.C. Liquid chromatographic assays for barbiturate injections, *J.Pharm.Sci.*, **1986**, *75*, 714–716.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 8.75
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, barbital, butobarbital, heptobarbital, hexobarbital, mephobarbital, phenobarbital, secobarbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70 adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 11.5
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, mephobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 50 μg/mL.

HPLC VARIABLES
Column: 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)
Mobile phase: MeOH:water 50:50
Flow rate: 0.6
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.09

OTHER SUBSTANCES
Simultaneous: aprobarbital, amobarbital, butabarbital, butalbital, secobarbital, thiopental, phenobarbital

REFERENCE

Forgács,E.; Cserháti,T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J.Chromatogr.A*, **1994**, *668*, 395–402.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 2 μBondapak C18
Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid
Flow rate: 0.27
Injection volume: 5
Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: butethal, butabarbital, talbutal, butalbital, amobarbital

KEY WORDS
mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.92 (A), 5.58 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine,

methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine +1 mL 500 mM pH 5.5 phosphate buffer, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL dichloromethane:isopropanol 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm Lichrospher 100 RP8
Column: 250 × 4 5 µm Lichrospher 100 RP8
Mobile phase: Gradient. MeCN:10 mM pH 4.4 phosphate buffer from 30:70 to 40:60 over 8 min, maintain at 40:60 for 6 min, to 30:70 over 1 min
Flow rate: 1
Injection volume: 20
Detector: UV 212

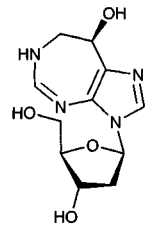
CHROMATOGRAM
Retention time: 11.1
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: barbital, allobarbital, butabarbital, phenobarbital, secobarbital
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, triflupromazine, trimethoprim, trimipramine

KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

Pentostatin



Molecular formula: $C_{11}H_{16}N_4O_4$
Molecular weight: 268.27
CAS Registry No.: 53910-25-1
Merck Index: 7277

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 20 μL isoamyl alcohol + 50 μL chloroform, vortex for 30 s, centrifuge at 20931 g for 10 min. Remove the aqueous layer and add it to 1 mL cold acetone (0°), vortex for 10 s, centrifuge for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 4:96 (Buffer was 5 mM sodium pentanesulfonate, pH 7.2.)
Column temperature: 40
Flow rate: 1
Detector: UV 250

CHROMATOGRAM

Retention time: 5.82

OTHER SUBSTANCES

Extracted: vidarabine
Noninterfering: chlorothiazide, cytosine arabinoside, guanine arabinoside, hydroxyzine, kanamycin, metaproterenol, nystatin, penicillin G, phenobarbital, prednisone, sulfamethoxazole, theophylline, trimethoprim, uracil arabinoside

REFERENCE

Bowman,D.B.; Kauffman,R.E. Reversed-phase high-performance liquid chromatographic method to determine vidarabine and hypoxanthine arabinoside in biological fluids, *J.Chromatogr.*, **1982**, *229*, 487–491.

SAMPLE

Matrix: fermentation solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Phenomenex C8
Mobile phase: MeCN:MeOH:50 mM $(HN_4)_2HPO_4$ 2.5:2.5:95 adjusted to pH 7.4 with phosphoric acid
Flow rate: 1.5
Detector: UV 258

CHROMATOGRAM

Retention time: 6.9

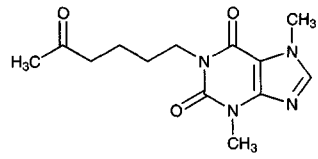
OTHER SUBSTANCES

Extracted: cytosine, coformycin, (8S)-pentostatin, 2'-deoxyguanosine, Ara-A

REFERENCE

Showalter,H.D.H.; Bunge,R.H.; French,J.C.; Hurley,T.R.; Leeds,R.L.; Leja,B.; McDonnell,P.D.; Edmunds,C.R. Improved production of pentostatin and identification of fermentation cometabolites, *J.Antibiot.(Tokyo)*, **1992**, *45*, 1914–1918.

Pentoxifylline



Molecular formula: $C_{13}H_{18}N_4O_3$
Molecular weight: 278.31
CAS Registry No.: 6493-05-6
Merck Index: 7278
Lednicer No.: 2 466

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM

Retention time: 11.477

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Add 6 mL ice-cold dichloromethane to 500 μL microsomal incubation, add 100 μL 10 μg/mL CT-2410 R, shake on a reciprocal shaker for 10 min, centrifuge at 3000 g for 10 min, evaporate the organic layer to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject 30-60 μL aliquot.

HPLC VARIABLES

Guard column: Opti-Guard (Optimize Technologies, Inc.)
Column: 100 × 4.6 3 μm Microsorb-MV C18
Mobile phase: MeOH:buffer 35:65 (Buffer was 25 mM ammonium phosphate containing 0.25% acetic acid, pH adjusted to 4.5 with ammonium hydroxide.)
Flow rate: 0.7
Injection volume: 30-60
Detector: UV 273

CHROMATOGRAM

Retention time: 9.3

Internal standard: CT-2410 R (20.0)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: lisofylline

KEY WORDS
human; liver

REFERENCE
Lee,S.H.; Slattery,J.T. Cytochrome P450 isozymes involved in lisofylline metabolism to pentoxifylline in human liver microsomes, *Drug Metab.Dispos.*, **1997**, *25*, 1354–1358.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

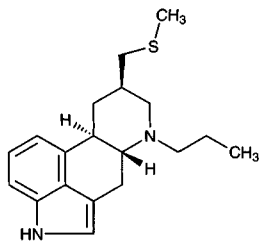
HPLC VARIABLES
Column: 150 × 6 5 μm 3-aminopropylsilyl silica gel with the amino group derivatized with 1,8-naphthalic anhydride (Bunseki Kagaku 1993, 42, 817)
Mobile phase: MeOH:buffer 50:50 (Prepare buffer by dissolving 6.183 g boric acid and 1.461 g NaCl in 500 mL water, adjust pH to 6.4 with sodium borate solution.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 20
Internal standard: 1,3-dimethyl-7-(2-hydroxyethyl)xanthine (12)

OTHER SUBSTANCES
Simultaneous: caffeine, hypoxanthine, propentofylline, theobromine, theophylline, uric acid, xanthine

REFERENCE
Nakashima,K.; Inoue,K.; Mayahara,K.; Kuroda,N.; Hamachi,Y.; Akiyama,S. Use of 3-(1,8-naphthalimido)propyl-modified silyl silica gel as a stationary phase for the high-performance liquid chromatographic separation of purine derivatives, *J.Chromatogr.A*, **1996**, *722*, 107–113.

Pergolide



Molecular formula: $C_{19}H_{26}N_2S$
Molecular weight: 314.49
CAS Registry No.: 66104-22-1, 66104-23-2 (mesylate)
Merck Index: 7304
Lednicer No.: 3 249

SAMPLE
Matrix: cell cultures
Sample preparation: Evaporate 100 μL 1 mg/mL lergotrile in MeOH in the bottom of a glass tube under a stream of nitrogen, add 2 mL culture homogenate (Polytron), add 2 mL 100 mM pH 8.5 sodium carbonate/sodium bicarbonate buffer, add 4 mL isoamyl alcohol, shake at 30 oscillations/min for 30 min, centrifuge at 1230 g for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250-500 μL mobile phase, filter, inject an aliquot.

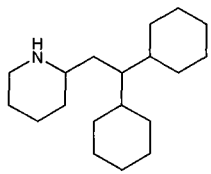
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.4 ammonium carbonate buffer 65.2:34.8
Flow rate: 2
Injection volume: 50
Detector: UV 290

CHROMATOGRAM
Retention time: 6.74
Internal standard: lergotrile (2.42)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Kerr,K.M.; Smith,R.V.; Davis,P.J. High-performance liquid chromatographic determination of pergolide and its metabolite, pergolide sulfoxide, in microbial extracts, *J.Chromatogr.*, **1981**, *219*, 317–320.

Perhexiline



Molecular formula: $C_{19}H_{35}N$
Molecular weight: 277.49
CAS Registry No.: 6621-47-2, 6724-53-4 (maleate)
Merck Index: 7305

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 200 μL 1.4 μg/mL hexadiline hydrochloride in 100 mM HCl + 50 μL 2 M pH 8.75 Tris-HCl buffer + 4 mL n-hexane, shake horizontally at 100 oscillations/min for 15 min, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 100 mM pH 10 sodium bicarbonate buffer, add 100 μL 5 mM dansyl chloride in acetone (freshly prepared), vortex, heat at 37° for 20 min, add 1.5 mL n-hexane, vortex, centrifuge at 10° at 2500 rpm for 3 min, freeze in dry ice/EtOH. Remove the organic layer and evaporate it to dryness at 60°, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 3 μm Velosep (Brownlee)
Mobile phase: MeOH:water 86:14
Column temperature: 45
Flow rate: 0.5
Injection volume: 25
Detector: F ex 366 em 470

CHROMATOGRAM
Retention time: 15.9, 16.8 (isomers)
Internal standard: hexadiline (19.5)
Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE

Morris,R.G.; Sallustio,B.C.; Saccoia,N.C.; Mangas,S.; Fergusson,L.K.; Kassapidis,C. Application of an improved HPLC perhexiline assay to human plasma specimens, *J.Liq.Chromatogr.*, **1992**, *15*, 3219–3232.

SAMPLE

Matrix: blood

Sample preparation: 400 µL Plasma + 400 µL 1 M diammonium hydrogen phosphate + 50 µL 2.5 µg/mL di-n-hexylamine in water + 100 µL 1 mg/mL diisopropylethylamine + 5 mL isopentane:dichloromethane 60:40, vortex for 1.5 min, centrifuge at 1500 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 100 µL 10 mg/mL trans-4-nitrocinnamoyl chloride in dry MeCN, vortex for 15 s, let stand at room temperature for 30 min, add 100 µL 25 mM sodium carbonate, vortex for 15 s, let stand at room temperature for 5 min, add 100 µL MeCN:50 mM ammonium acetate 50:50, vortex for 30 s, centrifuge at 1500 g for 5 min, inject a 130 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb phenyl
Mobile phase: MeCN:water:glacial acetic acid 46:53.5:0.5
Flow rate: 2
Injection volume: 130
Detector: UV 340

CHROMATOGRAM

Retention time: 18.4
Internal standard: di-n-hexylamine (10.2)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: dealkyldisopyramide, desethylamiodarone, desipramine, flecainide, mexiletine, nortriptyline, protriptyline, sotalol

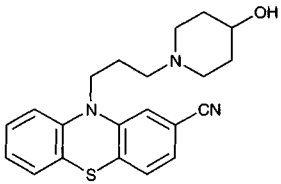
KEY WORDS

derivatization; plasma

REFERENCE

Grgurinovich,N. Method for the analysis of perhexiline and its hydroxy metabolite in plasma using high-performance liquid chromatography with precolumn derivatization, *J.Chromatogr.B*, **1997**, *696*, 75–80.

Pericyazine



Molecular formula: $C_{21}H_{23}N_3OS$
Molecular weight: 368.47
CAS Registry No.: 2622-26-6
Merck Index: 7306

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20